Questions:

1. Changes in expression of the enzymes of glycolysis and hydrogenosomal energy metabolism under increased iron growth conditions does not infer changes (increase or decrease) in ATP yield. Could the candidate comment on this statement.

2. It is striking that many of the critical proteins involved in hydrogenosomal metabolism are duplicated. Do you think gene duplication has a specific function and what is that?

3. As pointed out by the candidate in the introduction there are a variety of organelles that retain some features of the mitochondrion. Do you think this is the result of adaptation to an anaerobic environment with subsequent gain of cytochromes and a TCA cycle, or that the anaerobic organelle came first and adaptation to an aerobic metabolism resulted in the acquisition of cytochromes and TCA cycle.

4. How does the proposed loss of thioredoxin reductase in metronidazole resistant isolates by Leitsch et al., fit with your transcriptome data?

5. Your data elegantly demonstrates discreet changes in expression of many proteins, if you repeated these experiments under low (5%) partial pressures of oxygen what result would you predict?

The research extends our current understanding of the role of iron in the expression of hydrogenosomal proteins. The work is particularly challenging since *T. vaginalis* has the largest genome of any protist and has multiple gene duplications making this kind of analysis extremely difficult. The candidate has clearly acquired expertise with cutting edge technology in the area of proteomic analysis and the attached publications are an important contribution to the field of parasitology in general, and hydrogenosome-containing parasites in particular.

The candidate includes 5 publications in support of the degree:

2011 – PloS One - The minimal proteome in the reduced mitochondrion of the parasitic protist *Giardia intestinalis*. 11th author, 1 of 9 who did the experimental work.

This research provides valuable information concerning the proteome of G. intestinalis. A 139 candidate microsomal proteins are predicted from the study, of which 20 were experimentally confirmed as microsomal. Previous research had failed to identify any of these microsomal proteins. The work pushes the frontier of research with mitosomes forward and will be a much cited publication.

2011 - PLoS One - The core components of organelle biogenesis and membrane transport in the hydrogenosomes of *Trichomonas vaginalis*. 9th author, 1 of 8 who did the experimental work.

This research highlights similarities and differences in mitochondrial and hydrogenosomal membranes. The outer membrane posses a Tom 40 and a Sam 50 with different architecture to the mitochondrial membrane, while lacking a VDAC. The inner membrane exhibits the greatest differences, possessing a Tim 17/22/23, a PAM complex and an ADP/ATP carrier cassette. These studies provide the first real basis for understanding import machinery in the hydrogenosome.

2012 – MBP - Alternative 2-keto acid oxidoreductases in *Trichomonas vaginalis*: artifact of histochemical staining. 2nd author, no statement of contribution to data analysis or writing.

Demonstrates the absence of 2-keto acid oxidases in T. vaginalis, previously reported by Brown et al., 1999) to be important in metronidazole resistance.

2012 – GBE - Transcriptomic identification of iron-regulated and iron-independent gene copies within the heavily duplicated *Trichomonas vaginalis* genome. 5th author, no statement of contribution to data analysis or writing.

The effect of growth with and without iron on gene expression by the parasite. A number of enzymes in various pathways are reported, including glycolysis, Fe-S cluster assembly, hydrogenosomal membrane proteins, amino acid metabolism, etc. The results demonstrate a clear effect of iron on the regulation of hydrogenosomal energy metabolism; with malic enzyme and pyruvate ferredoxin oxidoreductase exhibiting the strongest iron-dependent upregulation. An interesting finding was that not all gene copies for these enzymes were upregulated suggesting selective gene expression by iron.

2013 – PloS One - Iron-induced changes in the proteome of *Trichomonas vaginalis* hydrogenosomes. 1st author, involved in manuscript preparation, experimental design, data analysis, and experimental work.

This study concentrates on iron induced changes in enzymes involved in hydrogenosomal energy metabolism. It complements the work published in GBE, 2012 and extends some of those observations. It is a valuable addition to the literature providing important information on paralogous gene expression under these conditions.

Only on the last publication (PLoS one, 2013) is it stated that the candidate played a role in all aspects of the research (experimental design, data analysis and writing). It is also unusual that the candidate has no work in press or in preparation since the date on the last publication is three years ago. It could be that the candidate because of lack of experience in publishing and possibly English not being the first language relied on the mentor for manuscript preparation. As it seems the candidate's role on many of the manuscripts was experimental, it is difficult to determine how much she contributed to the publication, especially where there are 11 authors.

The candidate has written an introduction and included a considerable amount of unpublished work as part of the thesis. The Introduction covers the background and clearly defines the goals of the thesis. This is reasonably well articulated; some minor suggestions are marked directly on the attached thesis that need to be addressed by the candidate.

Overall I would recommend the candidate for the award Ph.D.

Nigel Yarlett, Ph.D., F.R.S.C.
Distinguished Professor
Director of Haskins Laboratories
Director of the Graduate Program in Biochemistry